PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Craig A. Coburn et al.		Examiner: Yong Liang Chu
Serial No.:	10/534,291	Case No.: 21145YP	
Filed:	May 9, 2005		Art Unit: 1626
For:	PHENYLCARBOXAMIDE BETA-SECRETASE INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE		

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MING-TAIN LAI UNDER 37 C.F.R. § 1.132

I, Ming-Tain Lai, hereby declare as follows:

1. I am a citizen of the United States, and am over 21 years of age. A copy of my curriculum vitae is attached at Exhibit A.

2. In October 2002, HPLC assays of BACE1 (β-site amyloid precursor protein cleaving enzyme) were regularly conducted under my control and supervision at my laboratory at Merck's facility in West Point, Pennsylvania. Among the compounds tested were a series of phenylcarboxamide compounds, which were designed and synthesized by medicinal chemists working at Merck's West Point, Pennsylvania laboratories.

3. The BACE HPLC assay, which was a standard Merck assay, was developed by me and other biologists at Merck's West Point laboratories. The assay was designed to detect cleavage of a coumarin-labeled 10 mer peptide (coumarin-REVNFEVEFR), using either a Waters 2690 Alliance or Alliance HT HPLC instrument. The assay procedure is generally described in International application no. WO 2004/099376.

4. The BACE HPLC assays were conducted according to the following procedure. A reaction buffer was formed of the following ingredients:

MATERIAL	AMOUNT (µl)
4X NaOAc, 200mM, pH 4.5	25
BSA, 1mg/ml (Bovine Fraction V, Sigma #9647)	2.0
EDTA, 150mM, pH 4.5	10
10% CHAPS(Pierce, #28300)	2.0
Deferoxamine Mesylate, 50mM (Sigma, #D9533)	2.0
b-BACE1 (20nM, 20mM Tris, pH 7.2)	10
H_2O	31

5. 8 µl of compound (in DMSO) was added to 90 µl of the reaction buffer, and the resulting mixture was incubated at room temperature with shaking for 30 minutes.

6. Thereafter, 2 µl of the substrate coumarin-CO-REVNFEVEFR (50µM) (as described in WO 2004/099376) was added to the mixture. The resulting mixture was maintained at 25°C for 30 minutes with shaking. The reaction was quenched with 25µl of 1M Tris-HCl pH8.0.

7. Samples of the mixture were then centrifuged in a tabletop centrifuge at 15K. For analysis with the Alliance HPLC instrument, 60 µl of the supernatant was removed. For analysis with the Alliance HT instrument, 50 µl of the sample was passed through a filtration system (Millipore, 0.22 µm hydrophilic) prior to HPLC analysis.

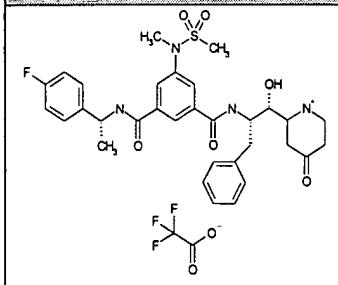
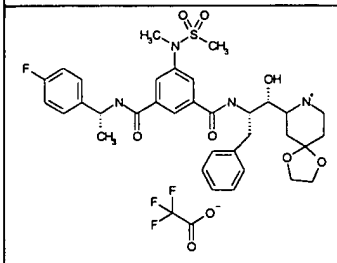
8. The HPLC conditions involved an Xterra RP18 column (3.5 µm, 2.1 x 150 mm). The mobile phase consisted of solvent A (0.05% trifluoro acetic acid in water) and solvent B (0.045% trifluoracetic acid in acetonitrile), according to the following gradient:

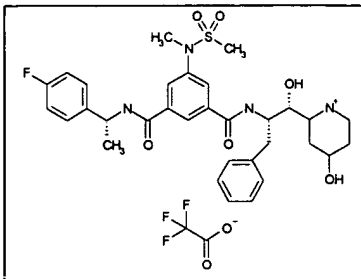
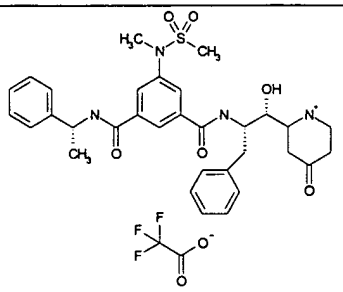
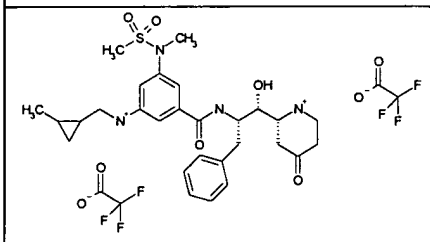
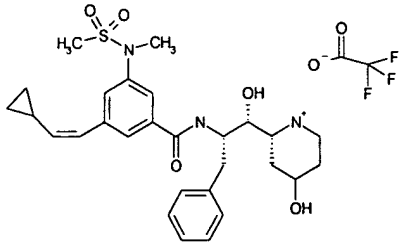
Time (minutes)	Percent Solvent B
0	19
3	25
4	95
5	19

Sample injection volumes were 50 µL for the Alliance and 25 µL for the Alliance HT. Detection was measured at 340 nm (excitation) and 440 nm (emission). Percent inhibition was measured according to the following formula:

(1-(area of product peak) of (E+S+compound)/area of product peak of (E+S)) x 100

9. The results of the HPLC assay for selected phenylcarboxamide compounds are set forth below:

COMPOUND	Date of Testing	Inhibition of BACE1 (nM)
	June 7, 2002	3
	June 7, 2002	220

	June 10. 2002	1
	July 9, 2002	3.5
	July 17, 2002	46
	October 7, 2002	11

10. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the instant application or any patent issued thereon.

Ming-Tain Lai

Dated: May 8, 2006

CURRICULUM VITAE OF MING-TAIN LAI

PERSONAL

A. Name: Ming-Tain Lai

B. Home Address: 52 Douglass Road
Lansdale, PA 19446

II. EDUCATION:

School	Dates	Major	Degree
Tunghai University Taiwan	1977-1981	Chemistry	B.S.
National Taiwan Normal University, Taiwan	1981-1983	Analytical Chemistry	M.S.
University of Minnesota	1987-1992	Bioorganic Chemistry	Ph.D.

III. MRL EMPLOYMENT HISTORY

Title	From - To
Research Fellow	8/30/01 - present
Senior Research Biochemist	8/30/95 - 8/30/01

IV. NON-MERCK EMPLOYMENT HISTORY

Postdoctoral Research Associate, 1992-1995
Massachusetts Institute of Technology
Supervisor: Professor JoAnne Stubbe

V. SOCIETY MEMBERSHIPS

American Chemical Society

VI. PUBLICATIONS IN PEER REVIEWED JOURNALS

1. **Lai, M-t.**; Shih, J-S., "Mercury (II) and Silver (I) Ion-Selective Electrodes Based on Dithia Crown Ether," *Analyst* **1986**, *111*, 891-895.

2. **Lai, M-t.**; Lin, W-M.; Chu, Y-H.; Chen, Y. S-l.; Kong, K-S.; Chen, C-w., "The Mechanism of Color Reversion in Soybean Salad Oil," *J. Am. Oil Chem. Soc.* **1989**, *66*, 565-571.

3. **Lai, M-t.**; Liu, H-w., " Inactivation of General Acyl-CoA Dehydrogenase by Enantiomerically Pure (Methylenecyclopropane)acetyl-CoA and Its Implication for This Enzyme-Catalyzed Reaction," *J. Am. Chem. Soc.* **1990**, *112*, 4034-4035.

4. **Lai, M-t.**; Liu, L-d.; Liu, H-w., " Mechanistic Study on the Inactivation of General Acyl-CoA Dehydrogenase by a Metabolite of Hypoglycin A,"*J. Am. Chem. Soc.* **1991**, *113*, 7388-7397.

5. **Lai, M-t.**; Oh, E.; Shih, Y.; Liu, H-w., "Synthesis of Enantiomerically Pure (Methylenecyclopropane)acetyl-CoA: the Causative Agent of Jamaican Vomiting Sickness," *J. Org. Chem.* **1992**, *57*, 2471-2476.

6. **Lai, M-t.** ; Liu, H-w., "New Evidence Supporting A Radical Mechanism of the Inactivation of General Acyl-CoA Dehydrogenase by a Metabolite of Hypoglycin," *J. Am. Chem. Soc.* **1992**, *114*, 3160-3162.

7. **Lai, M-t.**; Oh, E.; Liu, H-w., "Studies of the Inactivation of General Acyl-CoA Dehydrogenase (1*R*)- and (1*S*)-(Methylenecyclopropane)acetyl-CoA," *Bioorg & Med. Chem. Lett.* **1992**, *2*, 1423-1426.

8. **Lai, M-t.**; Li, D.; Oh, E.; Liu, H-w., " Inactivation of Medium-Chain Acyl-CoA Dehydrogenase by a Metabolite of Hypoglycin: Characterization of the Major Turnover Product and Evidence Suggesting an Alternative Flavin Modification Pathway," *J. Am. Chem. Soc.* **1993**, *115*, 1619-1628.

9. Thornburg, L.D.; **Lai, M-t.**; Wishnok, J. S.; Stubbe, J., " A Non-Heme Iron Protein with Heme Tendencies: An Investigation of the Substrate Specificity of Thymine Hydroxylase," *Biochemistry* **1993, *32*,** 14023-14033.

10. Horner, J. H.; Johnson, C. C.; **Lai, M-t.**; Liu, H-w.; Martin-Esker, A. A.; Newcomb, M.; Oh, E., " Highly Regioselective and Rapid Ring Opening of the (Methylenecyclopropyl)methyl Radical," *Bioorgan. & Med. Chem. Lett.* **1994**, *4*, 2693-2696.

11. Taoka, S.; Padmakumar, R.; **Lai, M-t.**; Liu, H-w.; Banerjee, R., " Inhibition of the Human Methylmalonyl-CoA Mutase by Various CoA Esters," *J. Biol. Chem.* **1994, *269*,** 31630-31634.

12. **Lai, M-t.**; Wu, W.; Stubbe, J., " Characterization of a Stable, Novel Adduct Resulting from the Inactivation of Thymine Hydroxylase by 5-Ethynyluracil," *J. Am. Chem. Soc.* **1995**, *117*, 5023-5030.

13. **Lai, M.-t.**; Di Cera, E.; Shafer, J.A., "Kinetic Pathway for the Slow to Fast Transition of Thrombin: Evidence of Linked Ligand Binding at Structurally Distinct Domains,"*J. Biol. Chem.* **1997**, *272*, 30275-30282.

14. Li, Y.-M.; Xu, M.; **Lai, M.-t.**; Huang, Q.; Castro, J. L.; DiMuzio-Mower, J.; Harrison, T.; Lellis, C.; Nadin, A.; Neduvelil, J. G.; Register, R.B.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shi, X.-P.; Yin, K.-C.; Shafer, J. A; Gardell, S. J., Photoactivated, active site directed γ-secretase inhibitors covalently label presenilin 1 *Nature*, **2000**, 405,689-694.

15. Li, Y.-M.; **Lai, M.-t.**; Xu, M.; Huang, Q.; DiMuzio-Mower, J.; Sardana, M. K.; Shi, X.-P.; Yin, K.-C.; Shafer, J. A.; Gardell, S. J., Presenilin 1 is Linked with β-Secretase Activity in the Detergent Solubilized State. *Proc. Natl. Acad. Sci. USA*. **2000**, 97, 6183-6143.

16. Shi, X.-P.; Chen E.; Yin, K.-C.; Na, S.; Garsky, V.; **Lai, M.-t.**; Li, Y.-M.; Platchek, M.; Regiater, B.; Sardana, M.; Tang, M.; Thiebeau, J.; Wood, T.; Shafer, J.; Gardell, S.J., The Pro Domain of ß-Secretase Does Not Confer Strict Zymogen-like Properties But Does Assist Proper Folding of the Protease Domain. *J. Biol. Chem.* **2001,** 276, 10366-10373.

17. Gardell, S.J.; Li, Y.M.; Xu, M.; **Lai, M.-t.**; Huang, Q.; Castro, J. L.; DiMuzio-Mower, J.; Harrison, T.; Lellis, C.; Nadin, A.; Neduvelil, J. G.; Register, R. B.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shi, X. P.; Yin, K.C.; Shafer, J. A., Photoactivated, active site directed γ-secretase inhibitors covalently label presenilin 1, in Alzheimer's Disease: Advances in Etiology, Pathogenesis and Therapeutics (Iqbal K, Sisodia SS and Winblad B eds) **2001**, pp 789-798, John Wiley & Sons, LTD, West Sussex, UK

18. Xu, M.; **Lai, M.-t.**; Huang, Q.; Castro, J. L.; Harrison, T.; Nadin, A.; Neduvelil, J. G.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shafer, J. A.; Gardell, S. J.; Li, Y. M., -Secretase: identification and Implication for Alzheimer Disease Therapy. *Neurobiology of Aging*, **2002**, 23, 1023-1030.

19. **Lai, M.-t.**; Chen, E.; Crouthamel, M. C.; DiMuzio-Mower, J.; Xu, M.; Huang, Q.; Price, E.; Register, R.; Shi, X. P.; Donoviel, D. B.; Bernstein, A.; Hazuda, D.; Shafer, J. A.; Gardell, S. J.; Li, Y. M. Presenilin-1 and -2 mediated γ-secretase activities are distinct for APP processing. *J. Biol. Chem.*, **2003**, 278 (25) 22475-22481.

20. Brady, S. F.; Singh, S.; Crouthamel, M. C.; Hollow, M. K.; Coburn, C. A.; Garsky, V. M.; Bogusky, M.; Pennington, M. W.; Vacca, J. P.; Hazuda, D.; **Lai, M.-t.**, Rational Design and Synthesis of Selective BACE 1 Inhibitors. *Bioorg. Med. Chem. Lett.*, **2004**, 14 (3), 601-604.

21. Coburn, C. A.; Stachel, S. A.; Li, Y.-M.; Rush, D. M.; Steele, T. G.; Chen-Dodson, E.; Holloway, K. M.; Xu, M.; Huang, Q.; **Lai, M.-t.;** DiMuzio J.; Crouthamel, M.-C.; Shi, X.-P.; Sardana, V.; Chen, Z.; Munshi, S.; Kuo, S.; Makara, G. M.; Annis, D. A.; Tadikonda, P. K.; Nash, H. M.; Vacca, J. P. Identification of a Small Molecule Non-Peptide Active Site ß-Secretase Inhibitor that Displays a Non-Traditional Binding Mode for Aspartyl Proteases. *J. Med. Chem.* **2004**, 47(25), 6117-6119.

22. Stachel, S. J.; Coburn,C. A.; Steele, T. G.; Jones, K. G.; Loutzenhiser, E. F.; Gregro, A. R.; Rajapakse, H. A.; **Lai, M.-t.**; Crouthamel, M.-C.; Xu, M.; Tugusheva, K.; Lineberger, J. E.; Pietrak, B. L.; Espeseth, A. S.; Shi, X.-P.; Chen-Dodson, E. C.; Holloway, M. K.; Munshi, S.; Simon, A. J.; Kuo, L.; Vacca, J. P. Structure-Based Design of a Series of Potent and Selective Cell-Permeable Inhibitors of Human ß-Secretase (BACE-1). *J. Med. Chem.* **2004**, 47(26); 6447-6450.

23. Shi, X.-P.; Tugusheva, K.; Bruce, J. E.,; Lucas, A.; Chen-Dodson, E.; Hu, B.; Wu, G., Price, E.; Register, R. B.; Lineberger, J.; Gate, A.; Miller, R.; Tang, M. J.; Espeseth, A.; Kahana, J.; Wolfe, A.; Crouthamel, M.-C.; Sankaranarayanan, S.; Simon, A.; Chen, L.; **Lai, M.-t.**; Pietrak, B.; DiMuzio, J.; Li, Y.-M.; Xu, M.; Huang, Q.; Garsky, V.; Sardana, M. K.; Hazuda, D. J. Novel Mutation introduced at the ß-site of amyloid precursor protein enhances ß- secretase cleavage in vitro and in cells. *J.Alzheimer's Dis.* **2005**, 7(2), 139-148.

24. Espeseth, A.; Xu, M.; Huang, Q.; Coburn,C. A.; Jones, K. L. G.; Ferrer, M.; Zuck, P.; Strulovici, B.; Price, E.; Wu, G.; Wolfe, A.; Lineberger, J.; Sardana, M. K.; Tugusheva, K.; Pietrak, B.; Crouthamel, M.-C.; **Lai, M.-t.**; Chen-Dodson, E.; Bazzo, R.; Shi, X.-P.; Simon, A.; Li, Y.-M.; Hazuda, D. J. Compounds that bind APP and inhibit Aß processing in vitro suggest a novel approach to Alzheimer Disease therapeutics. *J. Biol. Chem.* **2005,** 280 (18), 17792-17797.

25. Pietrak, B. L.; Crouthamel, M.-C.; Tugusheva, K.; Lineberger, J. E.; Xu, M.; DiMuzio, J. M.; Steele, T.; Espeseth, A.; Stachel, S. J.; Coburn, C. A.; Graham, S. L.; Vacca, J. P.; Shi, X.-P.; Simon, A.; Haduda, D. J.; **Lai, M.-t.** Biochemical and cell-based assays for characterization of BACE-1 inhibitors. *Anal. Biochem.* **2005**, 342, 144-151.

26. Stachel, S. J.; Coburn, C. A.; Steele, T. G.; Crouthamel, M.-C.; Pietrak, B. L.; **Lai, M.-t.**; Holloway, K.; Munshi, S. K.; Graham, S. L.; Vacca, J. P. Conformationally biased P3 amide replacements of ß-secretase inhibitors. *Bioorg. Med. Chem. Lett.*, in press.

27. **Lai, M.-t.**; Crouthamel, M. C.; DiMuzio, J.; Pietrak, B. L., M.; Donoviel, D. B.; Bernstein, A.; Gardell, S. J.; Li, Y. M.; Hazuda, D. A Presenilin- Independent Aspartyl Protease Prefers the β-42 Site Cleavage
J. Neurochem., **2006**, 96, 118-125.

VIII PATENTS

1. Nadin, A. J.; Neduvelil, J. G.; Sardana, M. K.; Shafer, J. A.; Gardell, S. J.; **Lai, M.-t.**; Li, Y.; Dorsey, B. D.; Dean, D. C., Investigational Compounds, Merck & Co., Inc. US Patent No. 6,753,410 B2, Granted Jun 22, 2004

2. Hazuda, D.; Dodson, E. C ; **Lai, M.-t** ;Xu, M. ;Shi, X.-P.; Simon, A, J. ; Wu, G. ;Li, Y. ;Register, R. B.,. Assays Using Amyloid Precursor Proteins with Modified Beta-Secretase Cleavage Sites to Monitor Beta-Secretase Activity. Filed in February, **2003.** A1 Published: 20031023 as US20030200555 A1

3. **Lai, M.-t.;** Crouthamel, M. C.; Brady, S. F., Beta-secretase Inhibitors, Application No. PCT/US03/15109, Filed May 14, 2003, Publication No. WO 03/099202 A2

4. Crouthamel, M. C.; Gardell, S. J.; Huang, Q.; **Lai, M.-t.**; Li, Y., Gamma-3 protease, Application No. PCT/US02/26969, Filed Aug. 8, 2002, Publication No. WO 03/018050 A1

IX. ABSTRACTS

1. Lai, M-t., Oh, E., Liu, L-d., Li, D., Liu, H-w., "Mechanistic Study on the Inactivation of General Acyl-CoA Dehydrogenase by a Metabolite of Hypoglycin A," XI Midwest Enzyme Chemistry Conference, University of Illinois, Chicago, IL, **1989.**

2. Lai, M-t., Oh, E., Liu, L-d., Li, D., Liu, H-w., "Mechanistic Study on the Inactivation of General Acyl-CoA Dehydrogenase by a Metabolite of Hypoglycin A" XI Midwest Enzyme Chemistry Conference, University of Illinois, Chicago, IL, **1990**.

3. Lai, M-t., Oh, E., Liu, L-d., Li, D., Liu, H-w., "Mechanistic Study on the Inactivation of General Acyl-CoA Dehydrogenase by a Metabolite of Hypoglycin A" XI Midwest Enzyme Chemistry Conference, University of Illinois, Chicago, IL, **1991**.

4. Lai, M-t., Li, D., Oh, E., Liku, H-w, "Mechanistic Study of the Inactivation of Medium-Chain Acyl-CoA Dehydrogenase (MCAD) by (methylenecyclopropyl)acetyl-CoA: Identification of a New Type of Flavin-inhibitor Adduct" XII Midwest Enzyme Chemistry Conference, University of Chicago, Chicago, IL, **1992**.

5. Li, D., Oh, E., Lai, M-t., Zhou, H-1., Becker, D.F., Stankovich, M.T., Liu, H-w., "Studies of the Inactivation of Short-Chain Acyl-CoA Dehydrogenase by Drivatives

of Methylenecyclopropaneacetyl-CoA" XIII Midwest Enzyme Chemistry Conference, Loyola University Chicago, ILL, **1993**.

6. Lai, M-t., Wu, W., Stubbe, J. "Mechanism Based Inhibition of Thymine Hydroxylase by 5-Ethynyl Uracil" American Chemical Society Meeting, Washington, DC, **1994**

7. Brady, S; Bruce, J.; Singh, S.; Crouthamel, M.-C.; Holloway, K. M.; Coburn, C.; Vacca, J. P.; Shafer, J.; Hazuda, D. " Development of BACE 1 Inhibitors" 9th International Conference on Alzheimer's Disease and related Disorders, Philadelphia, PA, July 17-22, **2004**

X. INVITED LECTURES

3/11/93	Department of Chemistry, National Chung-Cneng University, "Mechanistic Study of the Inactivation of Medium Chain Acyl-CoA Dehydrohegenase by (methylenecyclopropane)acetyl-Co-A"
3/15/93	Department of Chemistry, National Chiao-Tung University, "Mechanistic Study of the Inactivation of Medium Chain Acyl-CoA Dehydrogenase by (methylenecyclopropane)acetyl-Co-A"
3/18/93	Department of Chemistry, National Tsing-Hua University, "Mechanistic Study of the Inactivation of Medium Chain Acyl-CoA Dehydrogenase by (methylenecyclopropane)acetyl-Co-A"
3/22/93	Department of Chemistry, National Taiwan University, "Mechanistic Study of the Inactivation of Medium Chain Acyl-CoA Dehydrogenase by (methylenecyclopropane)acetyl-Co-A"
2/13/95	Department of Chemistry, National Taiwan University, "Characterization of a Stable, Novel Norcaradiene Adduct Resulting from the Inactivation of Thymine Hydroxylase by 5-Ethynyluracil"
2/16/95	Department of Life Science, National Tsing-Hua University, "Characterization of a Stable, Novel Norcaradiene Adduct Resulting from the Inactivation of Thymine Hydroxylase by 5-Ethynyluracil"
2/22/95	Department of Chemistry, National Taiwan Normal University, "Characterization of a Stable, Novel Norcaradiene Adduct Resulting from the Inactivation of Thymine Hydroxylase by 5-Ethynyluracil"
12/8/2003	Protease Targets and Drug Discovery Conference, Strategic Research Institute, "Development of BACE 1 Inhibitors"
7/21/2004	Press Release, 9th International Conference on Alzheimer's Disease and Related Disorders, "Development of BACE 1 Inhibitors"